

Ecophysiological studies on *Salvadora persica*

Abstract

The objective of this work was to study the ecophysiological response of *Salvadora persica* (*S. persica*) growing in Wadi El Gemal, to know the extent of the adaptation mechanism of the arak plant in its natural environment across the winter and summer seasons. Most physical and chemical characteristics of the soil supporting *S. persica* were significantly higher during 20-40 depth than during the 0-20 depth, but no significant differences were evident for pH and Mg^{2+} content between the two depths. Total chlorophyll, elements and chemical compositions studied of *S. persica* in the winter season were significantly higher than in the summer season, the opposite is true for total carotenoids, Mg^{2+} , S, P, total carbohydrate and proline contents. SDS-PAGE method was used to evaluate protein patterns in *S. persica*, which displayed different molecular weights of protein pattern across the winter and summer seasons. *S. persica* plants during the winter season showed a higher number of bands compared with those of the summer season. The number of polymorphic and unique bands was ten and three for *S. persica* leaves in both seasons, respectively. The differences in chemical compositions of *S. persica* due to seasonal changes led to the protein patterns changing and the activation of plant physiological stress tolerance mechanisms, indicating the protective role of these compositions and protein patterns in *S. persica*.

Key words: soil characteristics, phytochemical compositions, protein patterns, SDS-PAGE, *S. persica*.

1. INTRODUCTION

Ecophysiology is an experimental science to study the complex relationship between a plant's internal and external environments. Ecophysiologicals work to understand how organisms react to and make up for distinct external environmental stressors as well as to pinpoint physiological processes that serve as environmental adaptations [1]. Plant ecophysiology, also known as physiological plant ecology, is the study of how plants function and perform while subjected to environmental restrictions [2]. Plant ecophysiology provides a mechanistic understanding underlying current advances in the study of ecosystem fluxes [3] and plant community ecology [4]. Ecophysiological studies have been powerful in elucidating plant function and identifying traits that are adaptive in specific environmental conditions [5]. Plant ecophysiology deserves further study, particularly in light of projected changes in climatic conditions influencing ecophysiological function [2].

Desertification is an important issue in semi-arid climates, where soil erosion is increasing quickly and vegetation cover is steadily declining. Climate change is an important factor for sustainable water resource management in arid and semi-arid countries [6], and for modifying species distribution in desert areas controlling environmental heterogeneity and disturbances effects on plant species diversity [7]. Plant species have developed a variety of coping mechanisms to enable them to thrive and adapt in a variety of climates and terrains in response to drastic environmental changes [8]. Abiotic stressors have had a significant impact on the morphological, biological, and biochemical systems of plants. Two important environmental factors

that affect plant productivity and spread are drought and salinity [9]. In order to find and comprehend the mechanisms underlying drought resistance, there is growing interest in investigating the physiological behavior of different plant species [10]. Drought tolerance is accomplished through a variety of physiological and molecular methods, including osmotic adjustment, antioxidants, and scavenger compounds [11]. While, decreased stomatal conductance, reduced leaf area, and extensive root systems are morphological changes used to achieve drought avoidance in plants [12].

Salvadora persica L. (*S. persica*) is classified into the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, subclass Dilleniidae, order Capparales and family Salvadoraceae [13]. *S. persica* is frequently known also as Miswak, Siwak or Arak [14]. It is one of the most commonly widely used for teeth cleaning, oral hygiene (especially among the global Muslim community) and other medicinal properties, with great ethnobotanical importance [15,16]. In Islam, there has been an ascent in prophetic medicine, which derives its principles from all deeds and recommendations made or carried out by the prophet Muhammad, peace be upon him, and *S. persica* has been directed toward promoting health [17]. *S. persica* plant received WHO approval in 2000 as a practical, inexpensive toothbrush that may be used on a regular basis [18]. Pharmacological studies showed that the *S. persica* plant possesses alexiteric, analgesic, anti-inflammatory, anti-microbial, anti-plaque, astringent, diuretic aphrodisiac anti-pyretic, bitter stomachic activities, hypoglycemic, antiplasmodial, antiseptic and antiplaque actions [14,19,20].

Because soil and plants are interdependent, the pH, amount of organic matter in the soil as well as the type and nature of vegetation affect its physical, chemical, and biological characteristics [21,22,23]. The variations in the measured element concentrations are not explained by the nature of the soil in which the plants are grown, but rather by the interactions between the elements or the genotype of the plants [24]. Key physiological functions might be hampered and cellular architecture can be disrupted by environmental stress such as drought, salinity, and temperature [25], which can lead to turgor loss of plant species [26]. The organism's or the community's ability to adapt to its surroundings depends on all environmental influences acting on it at once, not just one of them. Additionally, the degree to which a certain component is present in the habitat and restricting plant growth affects how an organism or the physiognomy of the vegetation adapts [27]. To respond to stressful environmental conditions, the plants synthesize a vast array of metabolites [28,29]. El-Absy [29] noted that seasonal fluctuations have a considerable impact on the majority of the chemical components in the plant. This may be due to seasonal variations in physiological demands and effort rather than plant content availability [30]. Recent phytochemical investigations of *S. persica* different parts afforded different classes of compounds metabolites [31]. Many researchers such as [32-35] have used the method of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) in *S. persica*.

In this study, the main objective of research on the ecophysiology of *S. persica* plant is to know the extent of the adaptation mechanism of the arak plant in its natural environment and to the possibility of osmotic adjustment adopted to a tolerance of harsh environmental conditions during the winter and summer seasons.

2. MATERIALS AND METHODS

2.1. Study area

The largest wadi along the Egyptian Red Sea coast is Wadi El Gemal (Fig. 1). Wadi El Gemal National Park spans 4,770 km² of land and 2,000 km² of marine

waters, including more than 70 km of breathtaking shoreline. The Park extends from the Eastern Desert's sharp hills via a web of protected wadis to the Red Sea's emerald-colored depths. In Wadi El Gemal, 140 plant species were discovered, many of which are useful medicinally. The environmental conditions in Wadi El Gemal, which stretches from west to east, are the most diverse, including the types of soil, plant communities, and animal species assemblages [36,37]. The study location (24°33'45.9" N and 34°51'21.1" E) was visited from August 2019 (summer season) to January 2020 (winter season).



Fig. 1. Location map of Wadi El Gemal, Red Sea coast, Egypt.

2.2. Distribution and Description of *S. persica*:

S. persica is a desert perennial evergreen facultative halophytic plant [38], capable of growing under extreme conditions, from very dry environments to highly saline soils [39]. It is mainly distributed in dry and subtropical regions of Africa and the Middle East, as well as the Indian subcontinent [40] such as Saudi Arabia, Yemen, Iran, Iraq, Egypt, India, Pakistan, Malaysia, Sudan, Ethiopia, and Mauritania as well as countries in Central Africa, Southwestern Africa, and South America [39].

S. persica is a small, soft, light-yellow woody tree that branches to form a large leafy bush with a widely spreading crown, with a life span of 25 years [13], and its height is up to 10 m, with a diameter up to three feet [31]. Branches are numerous, drooping, glabrous, terete, finely striate, shining, and almost white. Leaves are somewhat fleshy, glaucous, 3.8–6.3 by 2–3.2 cm in size, elliptic-lanceolate or ovate, obtuse, and often mucronate at the apex, the base is usually acute, less commonly rounded, main nerves are in 5–6 pairs. The flowers are greenish yellow in color, in axillary and terminal compound lax panicles 5–12.5 cm long, numerous in the upper axils, pedicels 1.5–3 mm long, bracts beneath the pedicels, ovate and very caduceous [41]. Fruits are drupes with persistent calyx and corolla. They are fleshy, globose, single-seeded, smooth, 5–10 mm in diameter and spherical in shape. The fruits are pink to scarlet in color when mature [42].

2.3. Soil physical and chemical properties

Soil samples were collected from the three random points at the two depths 0–20 cm and 20–40 cm in the Wadi El Gemal. The soil samples were carried to the laboratory in closed tins to be used for soil physical and chemical analyses. Soil samples were air-dried, sieved and used for mechanical analysis of soil particles as

suggested by Jackson [43] and Rowell [44] for soil texture, and they are expressed as a percentage of the original weight. In this study, the soil moisture content was calculated according to the method described by Rowell [44]. The electrical conductivity (EC) and pH value for each sample were carried out using soil-water paste, according to Jackson [45], EC was expressed as ds/m^{-1} . The soil soluble cations (meq/L) including sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), as well as the soil soluble anions (meq/L) including chlorides (Cl^-) and bicarbonate (HCO_3^-) were determined using a saturation paste described by Tuzuner [46].

2.4. Plant analysis

The *S. persica* samples were manually taken in triplicates at random from the location under investigation in the winter and summer seasons of 2020 year. The samples were placed in plastic bags at the sites, then transferred immediately to the laboratory for preparation. Drying of collected plant materials was done in the oven at 70°C to a constant weight after which dried samples were milled to a fine powder and stored in brown bags at room temperature pending minerals and metabolites determinations.

The photosynthetic pigments parameters were quantified spectrophotometrically, and using the wavelengths of 663, 645 and 470 nm, the chlorophyll a (Chl.a), chlorophyll b (Chl.b) and total carotenoids were calculated by equations of Lichtenthaler [47], respectively. Atomic absorption spectrophotometry (GBC Avanta E, Victoria, Australia) was used to measure the concentrations of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), sulfur (S), Silica (Si) and phosphorus (P) [48]. The micro-Kjeldahl method was used to determine the total nitrogen (N) level [49].

The plant water content % is the difference between fresh weight (Wf) and dry weight (Wd) on the basis of fresh weight, and it can be calculated from the equation described by Jin et al., [50]; $\text{water content\%} = [(Wf - Wd) / Wf] \times 100$. According to Allen [51], the total protein percentage was calculated by multiplying the total nitrogen by 6.25. AOAC [52] procedures were used to calculate crude fiber. Based on the techniques of Chaplin and Kennedy [53] and Bates et al., [54], the contents of total available carbohydrates and proline were determined, respectively. The degree of succulence of the shoot systems of plants was calculated as the initial fresh weight / dry weight ratio (Fr.wt / Dry wt.) according to Dehan and Tal [55]. Samples of the shoot were weighed for fresh weight determination, then, washed with distilled water and dried in an aerated oven at 60°C until constant weight. Values of fresh weight (fr.wt.) and dry weight (dry wt.) were expressed in gram (g).

2.5. Protein Gel Electrophoretic:

The protein from four samples of *L. Shawii* leaves during both studied seasons and locations were isolated using a modified sequential extraction standard procedure developed by Curioni et al., [56].

- **SDS-PAGE Procedure:** Each sample was subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) by following the basic method developed by Laemmli [57] and modified by Singh and Shepherd [58]. The dried protein pellets were solubilized in 250 μL of a sample buffer. The electrophoresis was carried out using a 10% gel concentration [57]. A 10-well, 0.75 mm comb was used in a Bio-Rad Mini Protein 3 System having gel size 8.3–7.3 cm. The SDS-gels contained 4% polyacrylamide stacking gel and a resolving

gel of 10% polyacrylamide. Samples (30 μ L aliquots from sample (5 mg) extracted with 250 μ l of sample buffer) were applied into precast application slots.

- **Detection of protein Bands and Gel Imaging:** Upon the completion of electrophoresis, the proteins were fixed in methanol/acetic acid/water (40/10/50). Then staining with Coomassie Blue R-250. 200 ml of the destaining solution was used to destain the gel. The gel was gently agitated on a shaker for 2 hours. This destaining procedure was repeated several times until the background color of tile gel was removed. Total bands for each species were scored and their molecular weight (Mol. Wt.) calculated using the protein marker as standard. The gel scanning was done on Helena Jonior 24 photo scanner and the data were integrated using the scanner software. On the first well of each gel, the proteins were employed as the molecular weight (Daltons) markers ranging from 10–250 KDa.

2.6. Statistical analysis:

The Komolgorov-Smirnov test was used to confirm the normality of the data distribution. Then, the measured data were subjected to a two-way ANOVA test [59] and Student's t-test [60] to determine the statistically significant differences ($p \leq 0.05$ and $p \leq 0.01$) of the effect of soil depths and seasonal variations. The data of soil and plant analysis are expressed as the mean (\pm standard error) of three replicates. For a better understanding of the relationships between the qualities tested across experimental conditions, principal component analysis (PCA) was used. To perform statistical analyses in this study, the computer software program OriginPro 2018 b9.5.0.193 was used.

3. RESULTS AND DISCUSSION

3.1. Climatic conditions

The Wadi el Gemal region has a hot desert climate, as defined by the Köppen-Geiger classification. In Wadi El Gemal, the annual maximum temperature ranges from 23°C in January to 36°C in August. Rainfall totals 21 mm annually, with minimum amounts of 1 mm in July and maximum amounts of 5 mm in November. According to standards set by World Meteorological Organization [61], Wadi El Gemal is characterized by arid climates (< 150 mm). The lowest and highest percentage of relative humidity were registered in November and June of the 2020 year, respectively (Fig. 2). Temperature, as well as the strength and direction of the wind, have an impact on how much rain falls in the study region. One millimeter of rain every minute is the minimal quantity of rainfall that can result in runoff and flash floods, or around 10 millimeters overall [62].

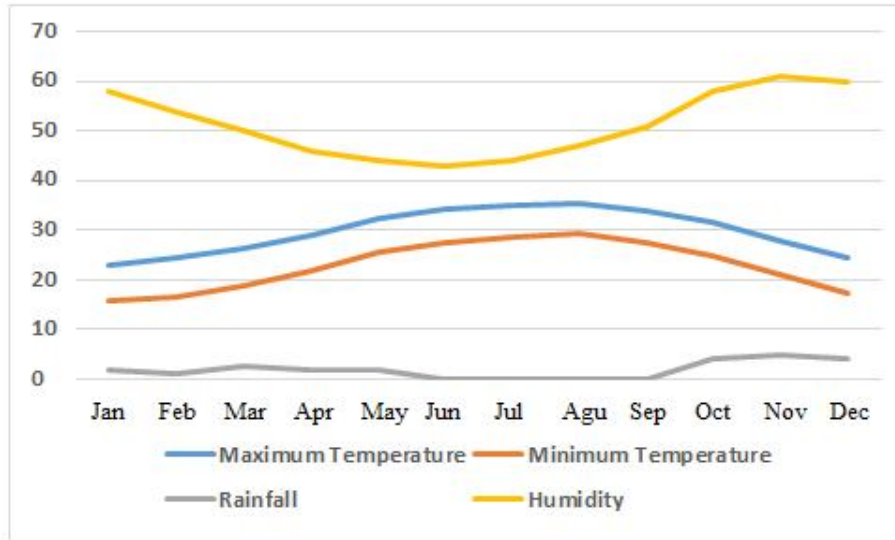


Fig.2. Monthly average temperature (°C), relative humidity (%) and rainfall (mm) in Wadi El Gemal during the 2020 year.

3.2. Soil variables

The physical characteristics of the soil supporting *S. persica* in the research location are shown in Table 1. All physical characteristics of the soil supporting *S. persica* differed significantly at probability 0.05 level between the two depths studied. The same finding was published by Salama et al., [63]. The ratios of sand, silt, and clay in soils taken from diverse environments reportedly varied significantly [64,65]. Significantly highest values of sand % were observed in 0-20 depth, while the clay and silt % showed the highest values in 20-40 cm depth at adjoining soil of *S. persica*. Based on the overall mean, the sand % was highest across the two soil depths compared with other soil particles, thus the soil of the studied location had a sandy texture. *S. persica* plants are found on clays, loam, black soils, and sand [66], and it is adapted to alkaline, non-saline, or very saline soils [67]. The texture of soil adjacent to *S. persica* varies from coarse (sandy) to fine (sandy-clay-loam) depending on the sampling site [68]. Sandy soil, also known as light-textured soil, has low water holding capacity due to rapid water infiltration and percolation, as well as high soil aeration [69].

Table 1. Mean values and standard error of mean (\pm SEM) for the mechanical properties % of the soil adjacent to *S. persica* across two depths.

Depths	Sand	Clay	Silt	Texture
0 – 20	92.00 \pm 2.12	5.17 \pm 1.15	2.83 \pm 0.48	Sandy
20 – 40	90.42 \pm 0.88	6.39 \pm 0.73	3.19 \pm 0.62	
Difference	1.58 \pm 2.30	-1.22 \pm 1.36	-0.36 \pm 0.78	
Overall	91.21 \pm 1.09	5.78 \pm 0.67	3.01 \pm 0.36	
P-Values	0.03*	0.02*	0.07*	

Statistically significant differences at $*p \leq 0.05$ between the two depths, according to Student's t-test.

In Egypt, the highest humid period of the year extended from November to April which was associated with low temperatures and evaporation; therefore, the soil had much favorable moisture [70]. As can be seen in Fig. 3, the results of the two-way ANOVA test showed that the depths, seasons, and their interaction had a highly

significant impact ($P < 0.01$) on the water content % in the adjoining soil of *S. persica*. The same tendency was observed by El-Lamey [71] and El-Absy [29] who reported that the water content % in the soil of plant species was significantly affected by depths and seasons during different habitat conditions. Of course, the water content % is significantly higher during the winter season than during the summer season, due to increase rainfall. The seasonal effect can result in favorable changes to the soil's water content, such as an increase in the availability of phosphorus needed for plant sustenance [72]. Additionally, it is significantly increased in 20 - 40 depth compared to 0 - 20 depth. Compared with the other interaction between depths and seasons, the interaction of 20-40 depth in the winter season recorded a significantly higher water content %, while, a significantly lower water content % was found in 0-20 depth in the summer season. Generally, the rhizospheric community can be affected directly or indirectly by soil characteristics including texture, pH, the presence of microaggregates, major cations, and organic matter [73]. The *S. persica* plant can live in harsh conditions and can tolerate soils ranging from intensely drought to highly saline [14,74]. The moisture gradient is complicated and linked to numerous environmental elements, including elevation, slope, climatic drought, soil texture, and character of the soil surface, according to Moustafa and Zayed [75].

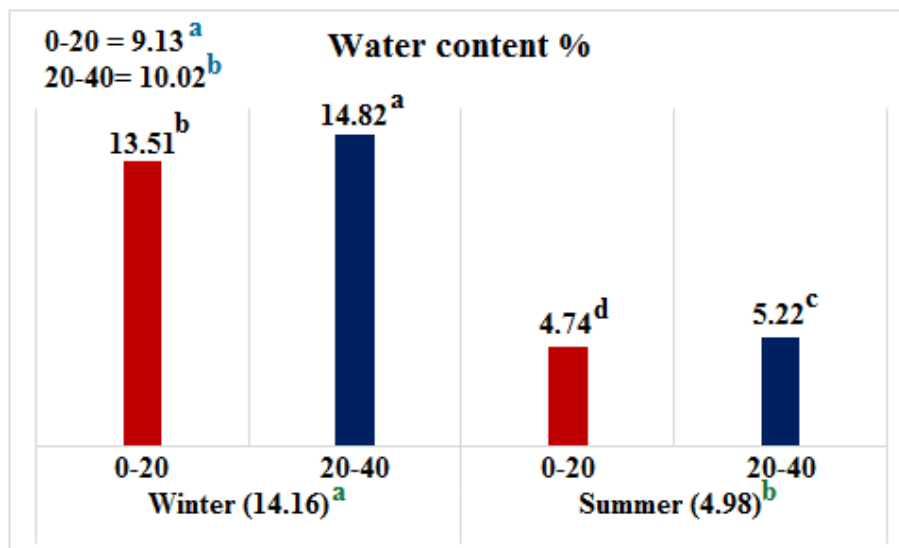


Fig. 3. Water content % at the soil associated with *S. persica* in the two depths and the two seasons during Wadi El-Gemal. Different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test.

The contents of Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Cl^- and other elements usually determine soil fertility. One of the most trustworthy and popular metrics, especially to describe field variability, is soil EC [76]. Statistically, the Student's t-test showed that all chemical characteristics of the soil associated with *S. persica* have significant differences ($P < 0.05$ or $P < 0.01$) between the 0-20 and 20-40 depths, except pH and Mg^{2+} properties, as shown in Table 2. In the study by Salama et al., [63], the measured chemical properties in the soil of *S. persica* showed significant differences ($p < 0.05$, 0.01). Significantly increased electrical conductivity (EC), Na^+ , K^+ , Ca^{2+} , Cl^- and HCO_3^- of the soil in the 20-40 cm depth were found compared with the 0-20 cm depth soil. While the increased pH and Mg^{2+} were noticed in the 0-20 and the 20-40 cm depths, respectively. The Na^+ , HCO_3^- and Ca^{2+} contents showed higher values relative

to the other chemical properties in the soil associated with *S. persica* at the two depths in the location studied, according to the overall mean. These results are similar to the analysis of adjoining soil of *S. persica* described by Aljeddani and Ahmed [76]. The variation in values of soil chemical properties between various environments may be due to location in relation to the distance from the sea and the types of surrounding mountains, both of which play a significant effect on soil characteristics [65]. The soil in the study area connected to *S. persica* tended to be alkaline based on the pH readings. At alkaline pH levels greater than 7.5, chemical interactions with some compounds, such as HPO_4 and CaCO_3 , cause the solubility of magnesium, calcium, and potassium as well as other elements to drop [77]. The soil adjacent to *S. persica* contains a large amount of carbonates that reflect its alkaline pH [76]. Decrease values of EC may have a substantial impact on plant health and nutrient availability because soluble salts are more likely to build in arid soils due to low precipitation and temperature rise, thus a high value of EC [78,79]. The values of EC range from 19.5 to 25.7 ds m^{-1} in the soil adjacent to *S. persica* [68]. The soil associated with *S. persica* in Wadi Gimal and its tributaries was soil rich in fine sand, pH and K^+ and poor in Mg^{+2} and water contents [63]. Significant variations in environmental factors are reflected in the variety of soil properties and texture [80]. The plants are able to adjust and flourish in environments with various soil characteristics [81].

Table 2. Mean values and standard error of mean (\pm SEM) for the chemical properties % of the soil adjacent to *S. persica* across two depths.

Depths	Ec (ds/m^{-1})	pH	Na^+ (meq/L)	K^+ (meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Cl^- (meq/L)	HCO_3^- (meq/L)
0 – 20	14.09 \pm 0.58	8.21 \pm 0.12	8.63 \pm 0.17	0.69 \pm 0.02	6.38 \pm 0.12	5.49 \pm 0.06	4.35 \pm 0.12	7.64 \pm 0.08
20 – 40	15.38 \pm 0.64	8.17 \pm 0.10	10.37 \pm 0.21	0.74 \pm 0.03	6.47 \pm 0.13	5.69 \pm 0.08	4.78 \pm 0.08	7.89 \pm 0.05
Difference	-1.29 \pm 0.86	0.04 \pm 0.16	-1.74 \pm 0.28	-0.05 \pm 0.03	-0.09 \pm 0.13	-0.20 \pm 0.09	-0.43 \pm 0.14	-0.25 \pm 0.10
Overall	14.74 \pm 0.48	8.19 \pm 0.07	9.5 \pm 0.41	0.72 \pm 0.02	6.43 \pm 0.08	5.59 \pm 0.06	4.57 \pm 0.11	7.77 \pm 0.07
P-Values	0.01*	0.81 ^{ns}	0.00**	0.02*	0.07*	0.11 ^{ns}	0.04*	0.06*

Statistically significant differences at * $p \leq 0.05$ and ** $p \leq 0.01$; ns: indicate the non-significant difference between the two depths, according to Student's t-test.

3.3. Plant variables

The alterations in photosynthetic pigments affect plant metabolism significantly [34], because they are vital components of the energy metabolism of plants [38]. Table 3 displays the photosynthetic pigment contents in *S. persica* during the winter and summer seasons. The photosynthetic pigment contents in *S. persica* were affected significantly ($P < 0.05$ or 0.01) by the two seasons. These photosynthetic pigment contents in *S. persica* plants indicate significant differences in response to changes in plant habitat [68]. Significant differences in all photosynthetic pigments in plant species between seasons were observed by El-Absy [29], Uvalle Saucedo et al., [82] and Devi et al., [83]. The winter season showed the highest levels of Chl.a, Chl.b, and Chl.a+b contents in *S. persica* plants. While, the highest values for Chl.a/b, total carotenoids and total pigment contents were observed in the summer season. The Chl.a content was greater than Chl.b content in *S. persica* plants, thus the values of Chl.a/b were more than 1 during the two seasons. Similar results were recorded for *S. persica* by Malhotra and Madan [84], and other species plants by [23,29,85]. During the two research seasons, *S. persica* plants had the greatest total carotenoids content, followed by contents of Chl.b and Chl.a, according to overall mean.

The increased concentrations of chlorophyll and carotenoids that desert plants achieved during dry conditions allowed the plants to adjust to variations in light

conditions and stresses [86]. According to Ait Said et al., [87], a decline in Chl.a can be viewed as a defensive adaptation mechanism that prevents increasing photon absorption. Each leaf area's chlorophyll content demonstrates how various plant species have adapted to the local environmental conditions [88]. Chlorophyll can substantiate the tolerance of plants to high pH conditions [89].

Table 3. Mean values and standard error of mean (\pm SEM) for the photosynthetic pigments contents (g/100g fr. wt.) of *S. persica* under winter and summer seasons.

Seasons	Chlorophyll a (Chl.a)	Chlorophyll b (Chl.b)	Total Chlorophyll	Chl.a/b	Total Carotenoids	Total Pigment
Winter	5.99 \pm 0.06	3.89 \pm 0.05	9.88 \pm 0.11	1.54 \pm 0.01	435.00 \pm 2.89	444.88 \pm 3.00
Summer	4.68 \pm 0.05	2.36 \pm 0.02	7.04 \pm 0.02	1.98 \pm 0.04	441.00 \pm 3.46	448.04 \pm 3.44
Difference	1.31 \pm 0.07	1.53 \pm 0.06	2.84 \pm 0.11	-0.44 \pm 0.04	-6.00 \pm 4.51	-3.16 \pm 4.56
Overall	5.34 \pm 0.29	3.13 \pm 0.34	8.46 \pm 0.64	1.76 \pm 0.10	438.00 \pm 2.42	446.46 \pm 2.16
P-Values	0.00**	0.00**	0.00**	0.00**	0.05*	0.03*

Statistically significant differences at * $p \leq 0.05$ and ** $p \leq 0.01$ between the two seasons, according to Student's t-test.

Using statistical analysis by Student's t-test, significant effects ($P < 0.05$ or 0.01) of seasons on all micro and macro elements compositions were found, except Ca^{2+} only (Table4). Accumulation of Na^+ and K^+ in *S. persica* plants showed significant differences in response to changes in plant habitat [68]. A significant seasonal trend was found in elements compositions of *S. persica* by Joshi et al., [90] and of other plant species by El-Absy [23], El-Absy [29], Al-Qahtani et al., [91], Kamel and El-Absy [92]. This may reflect seasonal changes in physiological needs and effort, rather than availability in plant content [30]. The contents of Na^+ , Ca^{2+} , Cl^- , Si, N and K^+ in *S. persica* plants in the winter season were higher than in the summer season, the opposite is true for other elements contents including Mg^{2+} , S and P. The change in mineral accumulation at various stands suggests that the plants have the capacity to control the uptake and accumulation of the elements from the external source in accordance with their adjustment needs, consequently, ions play a significant role in the production of osmotic potential in the xerophytes plants [24]. According to the overall mean during the two seasons, Cl^- and K^+ contents of *S. persica* plants registered the highest values compared with other micro and macro elements, followed by Ca^{2+} and levels, respectively. Similar to our results, Aljeddani and Ahmed [76] reported that the highest divalent component found is Ca^{2+} as well as the contents of K^+ and Na^+ are in equilibrium in *S. persica*.

The amount of nitrogen is the main factor that affects the leaf area, photosynthesis rate, growth and development of the plant. The Na^+ maintains the osmotic potential in the cells [93], and it is also related to physiological demands [94]. K^+ is a major plant macronutrient that plays important roles related to drought resistance [95]. *S. persica* depends highly on inorganic ions including Na^+ , Cl^- , and K^+ for osmotic adjustment and maintenance of cell turgor [68]. In *S. persica* plants, the effects of Ca^{2+} and Mg^{2+} contents on Na^+ efflux are transient, while they cause a prolonged shift toward K^+ uptake [38]. According to Salama et al., [96], stressed plants can modify their osmotic adjustment by absorbing and removing inorganic osmoregulatory ions including K^+ , Na^+ , Ca^{2+} , and Mg^{2+} . Si alleviates the toxic effects caused by salt stress, drought, and heavy metals [97].

Table 4. Mean values and standard error of mean (\pm SEM) for the micro and macro elements compositions of *S. persica* under winter and summer seasons.

Seasons	Micro elements ($\mu\text{g g}^{-1}$ dry wt.)						Macro elements (%)		
	Na ⁺	Ca ²⁺	Mg ²⁺	Cl	S	Si	N	P	K
Winter	1.74±0.02	1.48±0.05	0.99±0.05	2.45±0.03	1.00±0.01	0.52±0.01	2.18±0.06	0.31±0.01	2.53±0.02
Summer	0.89±0.03	1.40±0.01	1.53±0.02	1.25±0.04	1.21±0.03	0.49±0.01	1.36±0.05	0.37±0.02	1.85±0.03
Difference	0.85±0.04	0.08±0.05	-0.54±0.05	1.20±0.05	-0.21±0.03	0.03±0.02	0.82±0.08	-0.06±0.02	0.68±0.02
Overall	1.32±0.19	1.44±0.03	1.26±0.12	1.85±0.27	1.11±0.05	0.51±0.01	1.77±0.19	0.34±0.02	2.19±0.15
P-Values	0.00**	0.17 ^{ns}	0.00**	0.00**	0.00**	0.09*	0.00**	0.04*	0.00**

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference between the two seasons, according to Student's t-test.

The seasonal variations differences were statistically significant for levels of water content %, total protein, crude fiber ($P < 0.01$), total carbohydrate, proline and succulence degree ($P < 0.05$), as shown in Table 5. These results agreed with the results of Salama et al., [98] for water content, El-Absy et al., [26] for crude fiber and succulence degree, El-Absy [29] for total protein, Al-Qahtani et al., [91] for total carbohydrates, Tounekti et al., [68] for proline. The winter season of *S. persica* showed significantly higher levels of water content%, total protein, crude fiber, and succulence degree than the summer season. In contrast to the winter season, the summer season had higher levels of proline and total carbohydrates in *S. persica*. The proline and total carbohydrates can be used as an indicator of disturbed physiological conditions as drought and salinity stresses in most plant species [29,91,99]. Regarding the overall mean, the highest values had noticed for crude fiber, followed by total protein and total carbohydrate levels during the two seasons. Proline content in *S. persica* plants showed significant differences in response to changes in plant habitat [68].

To maintain the water content % in the mesophyll tissue of *S. persica*, the increase in epidermal thickness might be an adaptation of these plants to minimize the transpiration rate [38]. Thus, plants reduce their internal water potential under abiotic stresses [100]. Total soluble nitrogen may be contributing partially to the building up of the osmotic potential of plants [101], which may be accompanied by protein accumulation to improve the plant species to drought stress tolerance [102].

Under stress conditions, the total available carbohydrates are converted to soluble sugars that lead to decreasing osmotic potential, and variations in water potential gradient are responsible for water, organic and inorganic solute translocations in the plant cells [103]. Proline is an important osmolyte produced at a high cost of energy as compared to the uptake of Na⁺ into the cytoplasm, and it is increased under stress conditions [38]. Proline accumulation in the leaves and roots of *S. persica* seems to play a role in osmotic adjustment and osmo-protection [68]. Succulence degree is one of the most common features of many halophytes as well as xerophytes which can be increased by univalent more than by divalent or trivalent ions [26]. The increased leaf succulence in *S. persica* might be the plant's attempt to increase the size of its vacuole, and to counter the increased Na⁺ ion content from the cytosol of the leaf tissue, where sequester large quantities of Na⁺ away from metabolic active compartments of the cell [38].

The plant species seek to modify their internal osmotic pressure by accumulating inorganic and organic solutes in order to counteract the external stress in the arid environment [24]. The ability of plants to accumulate inorganic ions in high quantities inside their tissues is the most important mechanism to maintain the plant water potential more negative than the external medium in order to maintain the water uptake [104]. A combination of physiological (accumulation of Na⁺ and K⁺ in

the leaves) adjustments and increased osmo- (accumulation of proline) and photo- (reduction in chlorophyll content) protection helped the *S. persica* plants overcome salinity, hypoxia, and their combination in its native habitats [68]. Diverse mechanisms to adapt are supported by rich and complex metabolic networks that enable the plant to synthesize a wide range of compounds[8].

Table5. Mean values and standard error of mean (\pm SEM) for some chemical compositions of *S. persica* under winter and summer seasons.

Seasons	Water Content %	Total Protein	Crude Fiber	Total Carbohydrate(g%)	Proline (mg/g)	Succulence degree
Winter	54.69 \pm 0.64	13.65 \pm 0.38	39.28 \pm 1.80	9.58 \pm 0.07	5.62 \pm 0.07	1.78 \pm 0.03
Summer	40.73 \pm 1.15	8.52 \pm 0.31	28.16 \pm 1.10	10.81 \pm 0.55	7.60 \pm 0.49	1.57 \pm 0.04
Difference	13.96 \pm 1.32	5.13 \pm 0.49	11.12 \pm 2.11	-1.23 \pm 0.55	-1.98 \pm 0.49	0.21 \pm 0.05
Overall	47.71 \pm 3.18	11.08 \pm 1.17	33.72 \pm 2.66	10.20 \pm 0.37	6.61 \pm 0.49	1.68 \pm 0.05
P-Values	0.00**	0.00**	0.00**	0.09*	0.02*	0.01*

Statistically significant differences at $*p \leq 0.05$ and $**p \leq 0.01$; ns: indicate the non-significant difference between the two seasons, according to Student's t-test.

3.4. Protein Gel Electrophoretic:

The protein profiles and relative mobility of bands (RF) extracted from *S. persica* leaves during the winter and summer seasons are presented in Table6 and Fig.4. The results indicated that the number of the total bands in *S. persica* was 13 band as well as the molecular weight ranged from 17 to 97 kDa across the two seasons. Also during both seasons, the RF values of *S. persica* plants ranged from 0.18 to 0.92. According to the protein profiles using SDS-PAGE, the seasonal variations led to changes in the protein patterns, where the number of bands present are twelve and eleven in the winter and summer seasons, respectively. While the number of bands absent are one and two in the winter and summer seasons, respectively. Twelve protein bands were observed in extracts of *S. persica* leaves, which ranged from 24 to 67 kDa [32]. While molecular weights ranged from 14 to 20 kDa for five α -amylases from the *S. persica*[33]. The salt stress induces alterations in the protein profile in plants [104], where the high molecular weight proteins may be responsible for reducing Na^+ inflow and hence raising salt stress tolerance [106].

The all persistent bands and RF appeared in both seasons except the bands number 7, 8 and 12. In contrast to the summer season, *S. persica* plants in the winter season were distinguished with two 7 and 12 bands with molecular weight 49 and 29 kDa as well as with 0.51 and 0.85 RF values, respectively. While, the band No. 8 was found only in the summer season with molecular weight 46 kDa and 0.57 RF value. The bands No. 1 and 13 had recorded the highest and lowest molecular mass with values 97 and 17 kDa of *S. persica* in both seasons, respectively. These results indicate that different molecular weights of *S. persica* in both seasons. *S. persica* showed high genetic diversity due to 85 loci showing polymorphism out of 106 loci [107]. Out of thirteen bands, the *S. persica* plants were characterized by the presence of three unique and 10 polymorphic bands in this study. The average of polymorphic bands No. and monomorphic bands No. were 9.4 and 2.22, they also added no unique bands were observed for either the accessions of *S. persica*[107]. According to Kamel and El-Absy [92], *lycium showii* plants demonstrated the appearance of the majority of low molecular weight proteins is unique, in agreement with our results of *S. persica* plants.

Table6. SDS-PAGE of protein bands extracted from *S. persica* leaves growing in Wadi El Gemal during the winter and summer seasons.

Marker M.W(kDa)	Bands No.	RF	M.W (kDa)	Winter	Summer	Polymorphism
120	1	0.18	97	1	1	Polymorphic
	2	0.24	85	1	1	Polymorphic
85	3	0.31	76	1	1	Polymorphic
	4	0.35	73	1	1	Polymorphic
50	5	0.41	65	1	1	Polymorphic
	6	0.47	56	1	1	Polymorphic
	7	0.51	49	1	0	Unique
35	8	0.57	46	0	1	Unique
	9	0.59	39	1	1	Polymorphic
25	10	0.61	37	1	1	Polymorphic
	11	0.69	35	1	1	Polymorphic
	12	0.85	29	1	0	Unique
20	13	0.92	17	1	1	Polymorphic
			1	12	11	
			0	1	2	

kDa: kilo Dalton; **RF = Relative mobility of bands; (1): Presence of bands; (0): absence of bands.**

In this study, PCA analysis was used to identify the similarities and dissimilarities relationships among protein bands of *S. persica* leaves during the two seasons (Fig. 4). The first (PC1) and second (PC2) principal components explained 56.15%, and 43.85% of the total variance of protein bands, respectively. The PC1 and PC2 mainly distributed and distinguished protein bands of *S. persica* across the two seasons into three groups. These results are in harmony with Kamel and El-Absy [92], who reported that protein bands were divided into different groups based on the differences among them. The first group was related to PC1 and includes the protein bands number 7 and 12, which are strongly positively associated with the winter season. The second group is related to PC2, which includes protein band number 8 across the summer season. While the third group comprised the other protein bands of *S. persica* leaves in both seasons. Each group contained protein bands that were highly similar across both seasons, the opposite is true. These findings show variations in the protein patterns of *S. persica* leaves in both seasons. According to Kamel and El-Absy [92], seasonal changes have a considerable impact on controlling the expression of protein patterns in plants, which could be evidence of the adaptation of the plant to different stresses, also the protein patterns may be considered as key genetic markers of stress tolerance. In both favorable and unfavorable growth conditions, stress proteins, which make up a large portion of molecular chaperones, play a crucial role in maintaining cellular homeostasis [108]. Plant responses to abiotic stresses involve interactions and crosstalk between many molecular pathways [8]. Genetic identities are influenced both by polymorphic loci and the number of monomorphic loci [107].

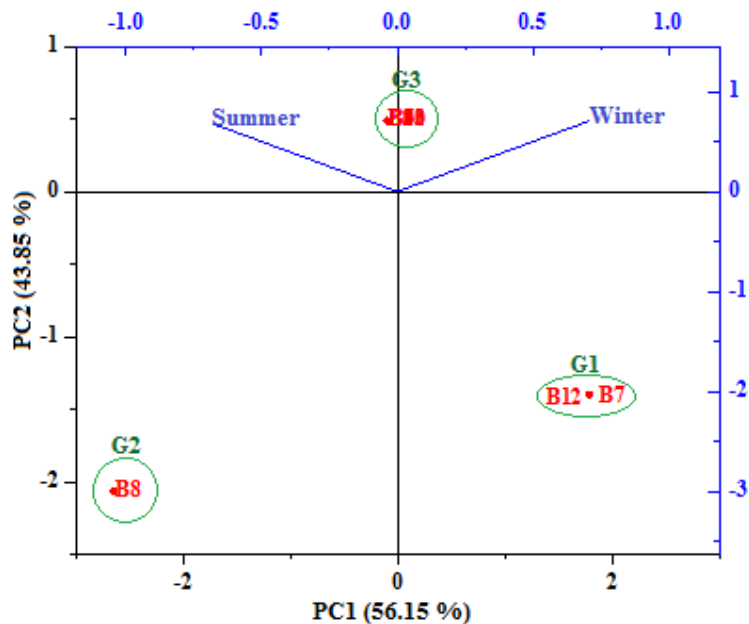


Fig. 4. Biplot diagram between PC1 and PC2 shows similarities and dissimilarities relationships between bands of *S. persica* during the two seasons. B1-B13: The bands No.1 – No. 13.

4. CONCLUSIONS

Significant differences in the chemical compositions of *S. persica* during seasonal changes indicate the maintenance of cellular osmotic balance to protect the plant during different stress conditions. Based on SDS-PAGE method, the protein patterns reflect variations of behavior and adaptation of *S. persica* under harsh environmental conditions. Thus, *S. persica* plants are favorable to the conditions of the arid desert. Generally, our results can provide information on the ecophysiological function and molecular characteristics of *S. persica* plants across seasonal changes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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